Differences Between Rose Cultivars in Susceptibility to Infection by *Botrytis cinerea*

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ABSTRACT

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Differences in the susceptibility of rose flowers to blossom blight caused by *Botrytis cinerea* were investigated. Rose flowers, cvs. Supra and Royalty, were inoculated with various concentrations of *B. cinerea* conidia and incubated in humidified chambers at 21 C. Disease severity was quantified 2 days later as the number of lesions that had developed on each flower. The slope of the inoculum concentration/disease severity (IC/DS) regression line was used as a measure of susceptibility. Supra was more susceptible than Royalty, but the susceptibility of each cultivar and the difference between them varied among sampling dates. In experiments using isolated petal disks, there was no difference between cultivars in the number of *B. cinerea* conidia that germinated on the petal surfaces. On several sampling dates, significantly fewer of the germinated conidia penetrated into Royalty petals, but differences in penetration were not always related to differences in susceptibility. Scanning electron microscopic examination of cryofractured petal disks suggested that the cuticle was important in preventing penetration. Cuticles isolated from Royalty petals were significantly thicker than those from Supra, which may account for some of the difference in susceptibility between cultivars. However, since differences in susceptibility were not consistently related to differences in penetration or cuticle thickness, other, unknown mechanisms were implicated.

Additional keywords: plant defense, resistance.

Botrytis cinerea Pers. is a widespread fungal pathogen that causes important diseases on many food and ornamental crops including a blossom blight on greenhouse-grown roses. Infection of the flowers causes direct damage to the harvested commodity of floral crops. Resistance to flower infections is also important for other crops since colonized petals frequently lead to latent infections that can destroy ripening fruit (4,5,10,23), serve as a source of conidia for secondary infections (13), or fall onto other plant parts and serve as a saprophytic base from which *B. cinerea* can invade other tissues that are not normally susceptible to infection by conidia (26).

There is undoubtedly a considerable range of resistance to *Botrytis* infection among the cultivars of greenhouse roses but it has been measured for only a few cultivars (15) and has not been characterized genetically. Pie and Brouwer (15) described differences in susceptibility to infection by *B. cinerea* among the highly susceptible rose cvs. Sonia, Madelon, and Melody and the more resistant cultivars Carambole, Gabriella, Pasadena, and Rubinette. They concluded that the lower disease severity (i.e., fewer and smaller lesions) on the more resistant cultivars was due to inhibition of postpenetration hyphal growth. Volpin and Elad (27) also described restriction of postpenetration growth of *B. cinerea* in rose petals. They reported that higher levels of tissue calcium significantly slowed disease progress (i.e., increase in lesion size) and suggested that the strengthening of cell walls and inhibition of pectolytic enzymes by calcium is the mechanism for restriction of infection.

This type of research is complicated by the fact that differences in resistance are small and quantitative in nature, making precise measurements of relative susceptibility critical. Furthermore, sus-

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ceptibility may change with the growing environment (8). In the present research, we sought to quantify the difference in susceptibility between two cultivars precisely and to identify morphological or physiological mechanisms of resistance.

MATERIALS AND METHODS

Experimental material. Supra and Royalty rose flowers (*Rosa hybrida* L.) were obtained from Dillon Floral Corporation, Bloomsburg, PA, or grown in a Department of Horticulture greenhouse at University Park. At Dillon Floral both cultivars were grown in the same greenhouse section on adjacent beds and received identical cultural practices. At University Park the roses were grown in 20-L plastic containers filled with Metro Mix 500 medium (Grace Sierra, Fogelsville, PA). All plants were irrigated twice each week with 180 mg/L N from 20N-4.3P-17K fertilizer (Peters 20-10-20, Grace Sierra) and leached once each week with water. The heating setpoint was 16 C and the ventilation fan setpoint was 25 C. Evaporative cooling pads were used from July through September of 1991.

The pots were arranged in four beds with 48 pots (24 of each cultivar) in each bed. To provide a nearly continuous supply of roses two of the beds were timed to flower approximately 3 wk later than the other two beds. Thus, for a given experiment roses were harvested from only two beds.

Roses were harvested at commercial maturity (sepals partially reflexed and outer two petals beginning to unfold) and stored until needed at 2 C in a hydrating solution containing, per liter of deionized water: 35.8 mg of 8- hydroxy quinoline hemisulfate, 81.5 mg of citric acid monohydrate, 441 mg of $CaCl_2 \cdot 2H_2O$, 1.47 g of $MgSO_4 \cdot 7H_2O$, and 4 mg of NaOCl.

B. cinerea was isolated from infected rose petals and grown on potato-dextrose agar at 20 C. Conidia were harvested from 13-day-old cultures and stored at −20 C on silica gel as described previously (21). To produce inoculum for experiments the stored conidia were seeded onto a medium containing, per liter: 1 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, 0.01 g of $FeSO_4 \cdot 7H_2O$, 2 g of L-asparagine monohydrate, 20 g of glucose, and 20 g of agar (14). The cultures were grown at 21±1 C under a 12-h photoperiod from a cool-white fluorescent lamp.

Inoculum concentration/disease severity (IC/DS) tests. Conidia were washed from 14-day-old cultures with autoclaved deionized water, strained through cheesecloth, vortexed with one drop (≅ 0.05 ml) of Tween-20, and centrifuged for 10 min at 2,000 g. The supernatant was discarded, the pelleted conidia were resuspended in autoclaved deionized water and spore concentration was determined with a hemacytometer. Spores were diluted in autoclaved deionized water.

Rose stems were recut 3 cm below the receptacle and individual flowers were inoculated with 0, 500, 1,000, 2,000, or 4,000 conidia per milliliter by spraying with a Preval spray unit (Precision Valve Corporation, Yonkers, NY). Each flower was sprayed for about 3 s to cover the outer petals with fine droplets. The flowers were incubated at 21 C in clear plastic crispers (30 cm L × 23 cm W × 10 cm D) with the stems submerged in deionized water and the flowers supported on wire racks above the water. The water maintained high humidity in the containers and water droplets were present on the flowers throughout incubation. After 2 days disease severity was determined by counting the number of lesions on each flower. At that time the lesions were clearly visible but had not yet coalesced.

The IC/DS tests were randomized complete block designs with two cultivars and five inoculum concentrations in a split plot arrangement. For each test there were at least five crispers, each containing five flowers of each cultivar (one per inoculum concentration). Each crisper constituted a block. Cultivar was the main plot factor and inoculum concentration was the subplot factor.

For each IC/DS test the following multiple linear regression model was fit:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2$$

where Y = lesion count, X_1 = inoculum concentration and X_2 is an indicator (dummy) variable for cultivar ($X_2 = 0$ for Royalty, $X_2 = 1$ for Supra). In this model b_0 and b_1 estimate the intercept and slope, respectively, of the IC/DS line for Royalty. The difference in intercepts between Royalty and Supra is estimated by b_2 and the difference in slopes is estimated by b_3. Regression functions for the individual cultivars can be written as follows:

$$\text{Royalty}(X_2 = 0)\text{: } Y = b_0 + b_1X_1$$
$$\text{Supra}(X_2 = 1)\text{: } Y = (b_0 + b_2) + (b_1 + b_3)X_1$$

The slopes of the IC/DS relationships were used as indices of the relative susceptibility of the flowers to infection (8).

Regression functions for the two cultivars could be fit separately, but combining the data for both cultivars into one model with an indicator variable facilitates statistical comparisons of the intercepts and slopes between cultivars (12). An F test for the hypothesis of $b_2 = 0$ is a test of the equality of intercepts. Similarly, a test of the hypothesis $b_3 = 0$ tests whether the two functions have equal slopes.

Germination and penetration tests. Two disks of tissue (1 cm diameter) were excised from each of the two outermost fully developed petals of a flower and placed, abaxial side up, on filter paper (Whatman 1) moistened with autoclaved deionized water. *B. cinerea* conidia were grown and harvested as described for IC/DS tests (above) and diluted to 10,000 spores per milliliter.

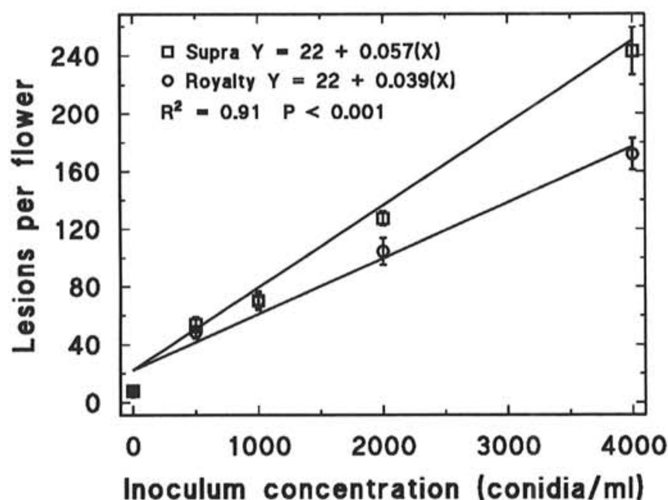

Fig. 1. Inoculum concentration/disease severity (IC/DS) relationships for Supra and Royalty rose flowers harvested 22 May 1990. Each point represents the mean of six flowers ± SE. The slope of the estimated regression function for Supra was significantly steeper than that for Royalty ($p < 0.001$), but the intercepts were not different ($P = 0.325$).

TABLE 1. Analysis of variance of regression for the inoculum concentration/disease severity (IC/DS) test on Supra and Royalty rose flowers harvested 22 May 1990

Source	df	Partial mean square	Partial F	$p(>F)$
Block	7	342	0.74	0.652
Cultivar[a]	1	480	1.04	0.343
Error a	7	464		
Inoculation	1	119,471	226	<0.001
Cultivar × inoculation[b]	1	13,386	25	<0.001
Error b	61	530		

[a]Indicator variable for cultivar tests the equality of the intercepts of the estimated regression functions of the two cultivars (i.e., hypothesis $b_2 = 0$).

[b]Inoculation by cultivar interaction tests the equality of the slopes of the estimated regression functions of the two cultivars (i.e., hypothesis $b_3 = 0$).

Each disk was inoculated with a 5-μl droplet of the spore suspension (i.e., ≈50 conidia) and incubated for 18 h at 21±1 C. We compared incubation times of 8, 12, 18, or 24 h in a preliminary experiment and found no significant differences in germination or penetration among the four incubation times (unpublished). We chose 18-h incubations for all subsequent experiments because it was easiest to judge penetration at that stage of infection.

After incubation the petal disks were killed and fixed in FAA (10 ml of 37% formaldehyde solution, 10 ml of glacial acetic acid, 100 ml of 50% ethanol). The fixed disks were rinsed once with deionized water then cleared with aqueous chloral hydrate solution (5 g of chloral hydrate plus 1 ml of deionized water). After clearing the disks were stained with 0.01% Trypan blue in lactic acid/glycerol/water (975:12.5:12.5 ml) and stored in 50% aqueous glycerol. Germination of conidia on the petal surfaces and penetration into the underlying tissue were determined by light microscopy at 400 magnification.

The germination and penetration experiments were two-factor nested designs. Treatment factors were cultivar (Royalty or Supra) and petal (first [outermost] or second). There were 12 flowers nested within each cultivar and two petals nested within each flower. Two replicates (disks) were inoculated from each petal. All statistical analyses were performed using SAS version 6.06.

Scanning electron microscopy. For cuticle surface observations petal disks were excised, inoculated, and incubated as described for germination and penetration experiments. After incubation disks were blotted dry with the corner of a tissue and the petal disks were affixed to aluminum stubs with double-sided adhesive disks. The mounted petal disks were fixed in the vapor from 25% (v:v) glutaraldehyde overnight and then air dried. The dried disks were coated with 42 nm of gold, then viewed and photographed on an ISI model 60 scanning electron microscope at 10 keV accelerating voltage.

For cryofracture cross sections petal disks were excised as described above and inoculated with 10-μl droplets of 40,000 *B. cinerea* conidia per milliliter. After incubation for 18 h at 21 C the disks were fixed in 3% (v:v) glutaraldehyde in 0.15 M sodium cacodylate buffer at pH 7.1, postfixed with 0.5% (v:v) OsO_4 in the same buffer, and dehydrated in a graded ethanol series. The dehydrated disks, in 100% ethanol, were frozen in liquid N_2 and fractured across the inoculation sites. Fragments were critical point dried, mounted on aluminum stubs, coated with 42 nm of gold, and viewed and photographed as described above.

Cuticle isolation. Petal disks were excised as described for germination and penetration experiments and floated, abaxial side up, on a solution of 9 units/ml pectinase and 2.5 units/ml cellulase in 50 mM sodium acetate buffer at pH 4.0. After 3–4 days at laboratory temperature, the abaxial cuticles were removed with forceps and the underlying tissue was discarded. The isolated cuticles were floated on enzyme solution for an additional 12–24 h to remove any adhering cellular debris, then rinsed three times with deionized water. Cuticles isolated in this manner were examined by SEM and found to be free of cellular debris. The rinsed cuticles were blotted on filter paper, transferred to tared vials,

TABLE 2. Relative susceptibility of Royalty and Supra rose flowers to infection by *Botrytis cinerea* compared with germination and penetration of *B. cinerea* conidia on petal disks

Source	Sampling date	Cultivar	Background infections[a]	Relative susceptibility[b]	Germination[c]	Penetration[c]
Dillon	7 Sep. 1989	Royalty	4.5 (4.2)	2.44	...[d]	...
Floral		Supra	0.9 (1.4)	3.51		
		$p(>F)$[e]		<0.001		
Dillon	19 Sep. 1989	Royalty	9.3 (5.2)	2.04	...	...
Floral		Supra	2.6 (2.5)	2.71		
		$p(>F)$		0.02		
Dillon	15 Nov. 1989	Royalty	...	...	28.1	12.9
Floral		Supra			29.9	20.1
		$p(>F)$			0.46	0.013
Dillon	19 Feb. 1990	Royalty	...	...	35.3	20.4
Floral		Supra			33.1	18.2
		$p(>F)$			0.30	0.33
Dillon	22 May 1990	Royalty	7.6 (2.8)	3.87	34.1	16.4
Floral		Supra	7.9 (7.2)	5.70	35.0	23.7
		$p(>F)$		<0.001	0.60	<0.001
Dillon	21 Aug. 1990	Royalty	5.0 (3.2)	2.87	33.2	19.2
Floral		Supra	2.5 (1.2)	3.50	31.8	17.4
		$p(>F)$		0.05	0.45	0.40
Dillon	5 Feb. 1991	Royalty	29.3 (9.4)	2.41	30.9	17.9
Floral		Supra	6.3 (3.3)	2.77	33.7	19.1
		$p(>F)$		0.27	0.14	0.57
PSU	12 Feb. 1991	Royalty	18.5 (17.3)	1.76	...	...
campus		Supra	8.7 (6.2)	2.54		
		$p(>F)$		0.003		
PSU	4 May 1991	Royalty	9.0 (3.0)	2.80	29.7	15.6
campus		Supra	6.4 (3.4)	3.91	32.3	21.1
		$p(>F)$		0.01	0.21	0.047
PSU	13 Aug. 1991	Royalty	6.5 (3.7)	2.97	36.2	7.5
campus		Supra	6.4 (5.1)	3.89	32.2	17.4
		$p(>F)$		0.03	0.04	<0.001
PSU	22 Oct. 1991	Royalty	37.5 (10.5)	1.61	30.4	17.5
campus		Supra	17.8 (6.1)	2.66	27.4	16.4
		$p(>F)$		0.002	0.14	0.61
PSU	14 Nov. 1991	Royalty	5.5 (4.9)	1.65	24.3	10.0
campus		Supra	5.7 (5.1)	3.86	26.9	12.0
		$p(>F)$		<0.001	0.02	0.11

[a] Number of lesions that developed on noninoculated flowers. (Flowers were sprayed with autoclaved deionized water before incubation.) Mean standard deviation of at least five flowers.
[b] Slope of the IC/DS relationship × 100.
[c] Number of conidia that germinated/penetrated (out of ≈50 applied) per inoculation site.
[d] Test not done.
[e] Significance of the F test for the null hypothesis of no difference between cultivars.

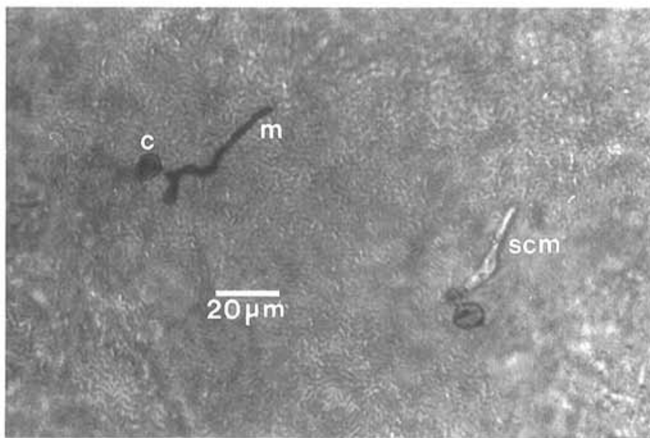

Fig. 2. Light microscopy of germinated *Botrytis cinerea* conidia on the surface of a rose petal 18 h after inoculation. The germling on the top has not penetrated the petal, thus the conidium (c) and surface mycelium (m) are stained darkly. The germling on the bottom has penetrated and the subcuticular mycelium (scm) is not stained.

dried to constant weight over anhydrous $CaSO_4$, and weighed to the nearest 0.01 mg. Weights were converted to micrograms per square cm and used as a measure of average cuticle thickness. Each vial contained six cuticle disks from a single flower (three from each of the outer two petals). Twenty flowers of each cultivar were used in each experiment.

Cuticles also were isolated from petal disks that previously had been fixed and counted in germination and penetration experiments (and subsequently stored in 50% aqueous glycerol). Twelve flowers of each cultivar were used in each experiment and each vial contained four cuticle disks.

RESULTS

Inoculum concentration/disease severity (IC/DS) tests. The results from a typical IC/DS test are shown in Figure 1. The intercepts of the estimated regression functions for the two cultivars were not different but the slope for Supra was significantly steeper than that for Royalty (Table 1). The relative susceptibilities (slopes of the IC/DS relationship $\times$ 100) for the two cultivars and the difference in susceptibilities varied among the sampling dates (Table 2). For each sampling date the relative susceptibility

Fig. 3. Scanning electron micrographs of germinated *Botrytis cinerea* conidia on the surfaces of rose petals 12 h after inoculation. **A,** The conidium (c) has produced a short germination tube terminating in an appresorium (a). **B,** A germling with more extensive surface growth but lacking an appressorium. **C** and **D,** Comparison of the surfaces of Royalty (**C**) and Supra (**D**) petals at equal magnification. Note the finer surface texture and presence of sunken areas on the Supra petals.

for Supra was higher than for Royalty. These differences were statistically significant ($P < 0.05$) for all except two sampling dates. Mean background infection levels (those infections not resulting from laboratory inoculation) varied, between cultivars and among sampling dates, from 2.4 to 37.5 lesions per flower (Table 2). The lesion counts on individual control flowers ranged from 0 to 52.

Germination and penetration tests. Conidia and mycelium on the surface of the petal disks stained dark blue, while mycelia growing under the cuticle were not stained, but were clearly visible (Fig. 2). Thus, both germination of the conidia and penetration by the germlings could be counted. There were no significant differences between petal positions in any experiment, so the data were pooled for presentation.

More than 75% of the conidia applied in infection droplets successfully germinated and attached to the petal surfaces (Table 2). For most sampling dates there were no significant differences between cultivars in germination of conidia on the petal surfaces. On one sampling date (13 August 1991) significantly more spores germinated on Royalty petals while on another date (14 November 1991) germination was higher on Supra.

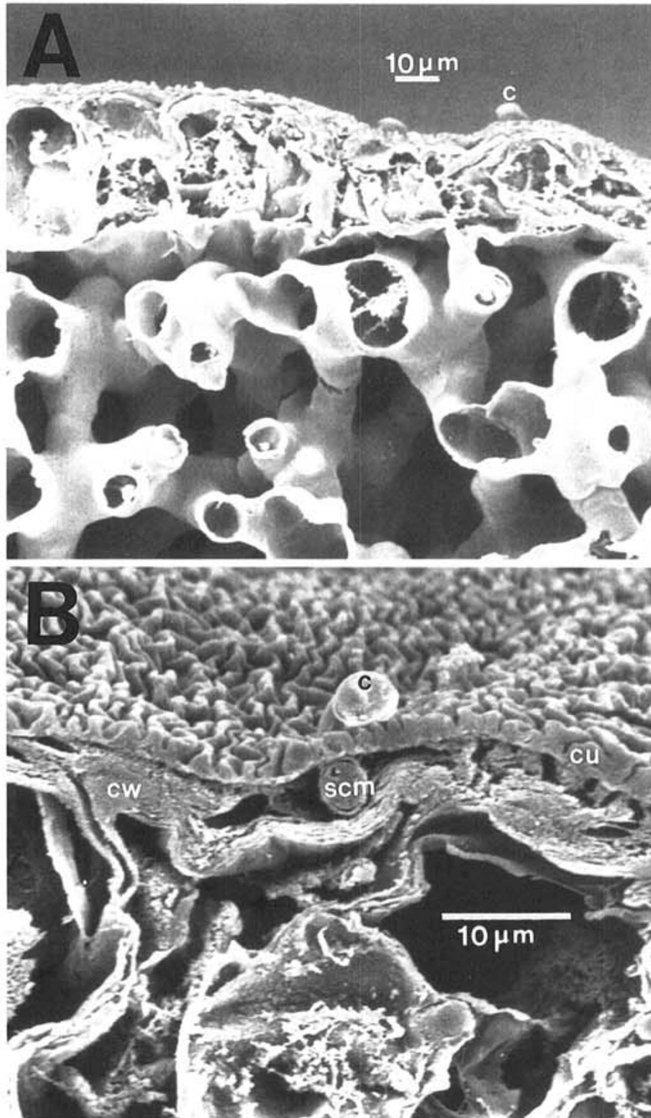

Fig. 4. Cryofracture cross sections of a rose petal 18 h after inoculation with *Botrytis cinerea* conidia. **A,** Epidermal cells under the inoculation site (right side of frame) have degenerated and collapsed. **B,** Cross section through a conidium (c) and associated subcuticular mycelium (scm). The cuticle (cu) over the infection site is intact, but the cell walls (cw) have swollen and split into several layers.

For sampling dates when there were no significant differences in relative susceptibility between the cultivars (as determined by IC/DS tests), there were no differences in penetration. On three dates when there were significant differences in relative susceptibility, significantly fewer germlings penetrated into the more resistant Royalty petals. However, on the remaining two dates when there were significant differences in susceptibility there were no differences in penetration between the cultivars (Table 2).

Scanning electron microscopy. The abaxial cuticle of the petals had a finely ridged surface (Fig. 3). Conidia germinating on the surfaces typically produced a short (1- to 2-μm) germ tube that terminated in an appressorium (Fig. 3A), although more extensive mycelial growth sometimes was observed (Fig. 3B) and not all germlings formed appressoria (Fig. 3B,C). The surfaces of the Supra cuticles were visibly finer in texture than those of Royalty and large (5- to 15-μm wide) furrows or depressed areas were frequently seen on Supra, but rarely on Royalty (Fig. 3C,D). These furrows also were visible under light microscopy on the cleared petal disks used for the germination and penetration tests. For example, for the 5 February 1991 sampling date (see Table 2) furrows were observed on 63% of the Supra disks but only on 11% of the Royalty disks.

At 18 h after inoculation the epidermal and some mesophyll cells under inoculation sites had degenerated and collapsed but the cuticles over the lesions remained intact (Fig. 4). The epidermal cell walls became swollen and split into several layers (Figs. 4B and 5B). The cuticle frequently separated from the epidermal cells and amorphous material was seen in the intervening space (Fig. 5A,B). Fungal mycelium was observed between the cuticle and cell walls (Figs. 4B, 5A,B), but there was no evidence of mycelia penetrating individual cells or of papillae formation by the host cells. When germlings failed to penetrate it appeared that penetration was blocked by the cuticle (Fig. 5C,D).

A furrowed area of a Supra cuticle also was examined. The cuticle in the furrow was thinner than in the surrounding area (Fig. 6).

Cuticle isolation. Cuticles isolated from Royalty petals were 30–67% thicker than those from Supra petals at each sampling date when cuticles were isolated (Table 3). For a given sampling date, cuticles isolated from fixed and cleared petal disks weighed considerably less than those from fresh petal disks.

DISCUSSION

Regression analysis of the IC/DS relationship provided a quantitative measure of the differences in susceptibility between cultivars. Uniform inoculum was applied to both cultivars and the flowers were incubated under controlled conditions. While background infection levels sometimes differed between cultivars and among sampling dates, such infections increased the disease severity at all inoculum levels. Thus, differences in background infection levels (between cultivars or among sampling dates) shifted the IC/DS line up or down. That is, the background infections affected the intercepts of the regression relationships but not the slopes. Thus the slope of the relationship is a measure of susceptibility that is independent of direct effects of the environment on the pathogen and not biased by naturally occurring inoculum levels. On a given sampling date, however, background infections did increase the variability in disease severity observed at each inoculum concentration. We did not attempt to control background infections since measures such as fungicide treatment or environmental manipulation could also affect host susceptibility (3,8).

The IC/DS tests established that Supra was more susceptible to infection than Royalty, but the susceptibilities of both cultivars and the differences between them were not constant over sampling dates. For a given sampling date the production environment and cultural practices were the same for both cultivars, hence observed differences in susceptibility are indeed due to genetic differences. Susceptibility to infection by *B. cinerea* in roses is known to change in response to environmental factors (3,8), but environmental data were not recorded over the course of the present experiments and no clear seasonal pattern is observable

Fig. 5. Cryofracture cross sections of rose petals 18 h after inoculation with *Botrytis cinerea* conidia (c). **A,** A germling has penetrated the cuticle (cu) and formed subcuticular mycelium (scm). **B,** The same area as in **A** viewed from a different angle. The cuticle (cu) has separated from the underlying cell walls (cw) which are swollen and disorganized. **C,** A germling that failed to penetrate the cuticle. **D,** The same area as in **C** viewed from a different angle.

in the susceptibility data. The fact that differences in susceptibility between cultivars varied among sampling dates suggests that the two cultivars respond differently to changes in the growing environment.

The infection process may be divided into four phases: germination of conidia on the petal surface, prepenetration growth, penetration into the host tissue, and postpenetration growth. The germination and penetration experiments were designed to determine which of these phases were inhibited in the more resistant Royalty petals. There was no difference between the cultivars in spore germination, but fewer of the germlings penetrated into Royalty petal disks. As with susceptibility measured in IC/DS tests, differences in penetration varied among sampling dates. While the contribution of other factors also must be considered, these data suggest that inhibition of penetration is one component of the greater resistance of Royalty petals to infection by *B. cinerea*. Inhibition of penetration (but not spore germination) also is responsible for the resistance of cucumber cotyledons (20) and broad bean and tulip leaves (7) to infection by *B. cinerea*.

In contrast to our results, Pie and Brower (15) reported only slight differences in conidial penetration of petals between cv. Sonia and the more resistant cvs. Carambole, Gabriella, and Pasadena and concluded that differences in penetration were not responsible for differences in susceptibility. They attributed the slower disease progress in the more resistant cultivars to the restric-

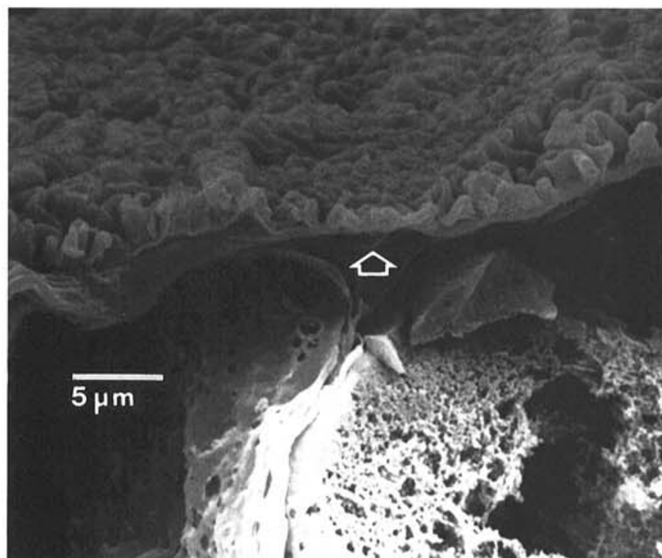

Fig. 6. Cryofracture cross section through a furrow (arrow) in the cuticle of a Supra rose petal.

TABLE 3. Weights of cuticle disks isolated from Royalty and Supra rose petals, compared with the relative susceptibility of the flowers to infection by *Botrytis cinerea* and penetration of *B. cinerea* germlings into petal disks

Source	Sampling date	Cultivar	Relative susceptibility[a]	Penetration[b]	Cuticle weight[c]	
					Fixed	Fresh
Dillon	22 May 1990	Royalty	3.87	16.4	0.13	...[d]
Floral		Supra	5.70	23.7	0.10	
		$p(>F)$[e]	<0.001	<0.001	0.023	
Dillon	21 Aug. 1990	Royalty	2.87	19.2	0.10	...
Floral		Supra	3.50	17.4	0.07	
		$p(>F)$	0.04	0.40	<0.001	
PSU	22 Oct. 1991	Royalty	1.61	17.5	0.13	0.22
campus		Supra	2.66	16.4	0.07	0.16
		$p(>F)$	0.002	0.61	<0.001	<0.001
PSU	14 Nov. 1991	Royalty	1.65	10.0	0.17	0.20
campus		Supra	3.86	12.0	0.10	0.12
		$p(>F)$	<0.001	0.11	<0.001	<0.001

[a]Slope of the IC/DS relationship × 100.
[b]Number of conidia that penetrated (out of 50 applied) per inoculation site.
[c]Fixed cuticles were isolated from the disks used in the germination and penetration tests. Fresh cuticles were isolated from petals of fresh roses that were harvested on the same sampling date. Units are $\mu g/cm^2$.
[d]Test not done.
[e]Significance of the F test for the null hypothesis of no difference between the cultivars.

tion of postpenetration hyphal growth. We also observed differences in susceptibility (as determined by IC/DS) when there were no differences in penetration, suggesting that postpenetration growth of the pathogen was inhibited in the more resistant cultivar. We did not quantify postpenetration hyphal growth in the present research, but inhibition of postpenetration hyphal growth is a common mechanism of resistance to infection by *B. cinerea* in other pathosystems (7,18,24).

While we saw no evidence for the restriction of infection hyphae in the cell walls of rose petals in the present histological studies, we used relatively high spore concentrations in both the germination and penetration and the cryofracture experiments, as did McKeen (11) and Pie and De Leeuw (16) in their histological studies. The combined action of infection hyphae from many germlings may have overcome host defenses that restrict postpenetration growth when inoculum densities are lower. Such a situation was described for the resistance of broad bean to chocolate spot disease caused by *B. fabae* (28).

Cell walls have been described as important barriers to infection by *B. cinerea* for tomato fruits (26), tulip leaves (7), reed canarygrass (25), and wheat (17), but they did not appear to contribute significantly to the inhibition of penetration in the present research. In fact, it appeared that direct penetration of host cell walls was not needed for successful infection. Cryofracture observations revealed the fungal mycelium to be growing between the cuticle and cell wall with cell death and extensive degradation of the cell walls occurring in advance of the fungal mycelium. The failure of many germlings to penetrate even when neighboring germlings had degraded the cell walls under the entire infection droplet suggested that the cell walls were not an important component of the resistance of petals to penetration.

These results are consistent with the histopathology of *B. cinerea* infections on Sonia rose petals as described by Pie and De Leeuw (16). They reported no evidence of papilla formation or other active defense reactions. Penetration through the cuticle occurred within 8 h after inoculation and small white lesions were visible macroscopically within 8–14 h. Although little subcuticular fungal development had occurred up to 16 h after inoculation, extensive degradation of the host cell walls and separation of the cuticle from the walls were observed. The first penetrations of epidermal cell lumina were not reported until 16–24 h after inoculation, several hours after lesions were visible macroscopically. Our observations and those of Pie and De Leeuw also are similar to those reported for the infection of broad bean leaves by *B. cinerea* (11) and the infection of onion leaves by *B. cinerea* or *B. squamosa* (2).

The cuticle also is an important barrier to penetration by phytopathogenic fungi in general (9,19,22) and by *B. cinerea* in particular (1,6,18,26). In the present study many *Botrytis* germlings failed to penetrate and form subcuticular hyphae even though the host cell walls under the entire infection drop were extensively degraded by nearby successful germlings. Large numbers of unsuccessful penetration sites were not examined in cross section, but our observations suggested that penetration was blocked by the cuticle. Cuticles of the more resistant Royalty were thicker than those of Supra, but differences in relative susceptibility and penetration were not always related to differences in cuticle thickness. Thus, it is not clear whether cuticle thickness is a primary determinant of the difference in resistance between cultivars. Still, the cuticle does appear to be an important barrier to infection in this pathosystem since, on Royalty petals, 40% or more of the conidia that germinated and attached to the surface failed to penetrate and establish infections. Histological evidence indicated that the cuticle was responsible for the inhibition of penetration, but the precise mechanism(s) of this resistance remains unclear.

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